

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/155, A61P 3/04, 3/10, 37/00		A1	(11) International Publication Number: WO 00/53171 (43) International Publication Date: 14 September 2000 (14.09.00)
(21) International Application Number: PCT/EP00/01849 (22) International Filing Date: 3 March 2000 (03.03.00) (30) Priority Data: FI99A000040 5 March 1999 (05.03.99) IT FI99A000215 19 October 1999 (19.10.99) IT (71) Applicant (for all designated States except US): MOLTENI L. E C. DEI FRATELLI ALITTI SOCIETA' DI ESERCIZIO S.P.A. [IT/IT]; Strada Statale 67 - Tosco-Romagnola, Localita' Granatieri, I-50018 Scandicci (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): MANNUCCI, Edoardo [IT/IT]; Via Dante 5, I-59100 Prato (IT). ROTELLA, Carlo, Maria [IT/IT]; Via S. Martino 6, Loc. Grassina, I-50012 Bagno A Ripoli (IT). OGNIBENE, Agostino [IT/IT]; Piazza Villamagna 5, I-50012 Bagno A Ripoli (IT). (74) Agent: GERVASI, Gemma; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(54) Title: USE OF METFORMIN IN THE PREPARATION OF PHARMACEUTICAL COMPOSITIONS CAPABLE OF INHIBITING THE ENZYME DIPEPTIDYL PEPTIDASE IV			
(57) Abstract The use of metformin in the preparation of pharmaceutical compositions useful for inhibiting the enzyme dipeptidyl peptidase IV, in particular for increasing the plasma concentration of Glucagon-Like Peptide-1, is described.			

BA

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

USE OF METFORMIN IN THE PREPARATION OF PHARMACEUTICAL
COMPOSITIONS CAPABLE OF INHIBITING THE ENZYME DIPEPTIDYL
PEPTIDASE IV

Field of invention

- 5 The present invention refers to the use of metformin in the preparation of pharmaceutical compositions useful for the treatment of pathologies requiring the inhibition of the enzyme dipeptidyl peptidase IV, in particular for increasing the plasma concentration of Glucagon-Like Peptide-1.

State of the art

- 10 It is known that the enzyme dipeptidyl peptidase IV (DPP-IV) is an enzyme present in the serum and expressed on the surface of endothelial cells in different parts of the body. This enzyme inhibits the action of Glucagon-Like peptide 1 (GLP-1), a hormone which stimulates insulin secretion and inhibits food intake, and of other hormones and neuropeptides including neuropeptide Y (NPY) and peptide YY
15 (PYY). While the actions of the enzyme have not been completely characterised, it is known that DPP-IV is involved in the modulation of immune responses (it is known by immunologists as CD26).

- Considering the wide spectrum of activity of DPP-IV, a product capable of inhibiting its activity could be very useful in the treatment of pathologies caused by
20 a deficit (relative or absolute) of hormones degraded by the enzyme or by an excessive activity of CD26.

It is also known that Glucagon-Like Peptide-1 (GLP-1) is an hormone produced by the endocrine cells dispersed in gastrointestinal mucosae (Orskov et al. Endocrinology 119:1467-75 (1986)). This hormone is secreted mainly after meals

rich in carbohydrate (Shima et al. *Acta Endocrinol Copenh* 123:464-70 (1990)) and has two main effects:

- a) it stimulates glucose-induced insulin secretion (Kreymann et al. *Lancet* 2:1300.1304 (1987)) and therefore is, at least partially, responsible for the
5 increase of insulin secretion in the early post-prandial phase;
- b) it inhibits food intake, through a direct action on the central nervous system (Turton et al. *Nature* 369:69-72 (1996)).

Since the hormone is capable of crossing the blood-brain barrier (Orskov et al. *Diabetes* 45:832-35, (1996)), the peripheral administration of the hormone
10 determines a stimulation of central GLP-1 receptors involved in the regulation of food intake; therefore, it is conceivable that the gastroenteric post-prandial secretion of GLP-1 contributes to the induction of satiety after a meal.

The use of exogenous GLP-1 in therapy by parenteral (subcutaneous or intravenous) or trans-mucosal administration in type 2 diabetes has been widely
15 investigated (Gutniak et al. *Diabetes Care* 20:1874-79 (1997), Rachman et al. *Diabetologia* 40:205-211 (1997)). The administration of exogenous GLP-1 during the meal is carried out in order to increase the secretion of insulin in the early post-prandial phase, correcting the insulin deficiency occurring in that phase in type 2 diabetes. An advantage of this therapy is that it also induces, facilitating body
20 weight control. A product capable of increasing the post-prandial concentration of endogenous GPL-1 (increasing its secretion or reducing its inactivation) would therefore be useful in the treatment of type 2 diabetes and obesity. The inhibition of DPP-IV has been considered as a treatment option for type 2 diabetes, and

various studies are at present carried out in order to identify pharmaceuticals possessing such activity.

Type 2 diabetes mellitus is a disease characterised by a reduced sensitivity to insulin action (insulin resistance), associated with insufficient insulin secretion, particularly in the early post-prandial phase. In different patients one or the other pathogenic component can prevail: in general, in obese patients insulin resistance is considered the main pathogenetic mechanism, while in normal weight subjects with type 2 diabetes the deficit in insulin secretion is more evident and the insulin resistance less marked. These differences requires different therapeutic approaches: in obese patients drugs such as metformin, which increase insulin sensitivity, are preferred, while in normal weight molecules capable of stimulating insulin secretion (such as sulfonylureas) are more often used.

Metformin is an oral hypoglycemic of the biguanide class, widely used as a first-approach therapy in overweight patients with type 2 diabetes.

The compound also shows a modest anorexic action; therefore, long-term metformin treatment usually reduced body weight, or prevents weight gain, in overweight type 2 diabetic patients.

Detailed description of the invention

It was now surprisingly found, and it is an object of the present application, that metformin, in the pharmaceutical form of administration usually employed and commercially available, inhibits the activity of DPP-IV and therefore can be useful for the preparation of pharmaceutical compositions to be used when the inhibition of such enzyme is requested.

In particular, and this is a second object of the present invention, it increases the plasma concentration of GLP-1, by stimulating hormone secretion and/or inhibiting its inactivation; metformin is therefore useful in the treatment of all the pathologic conditions where a deficit of GLP-1 is involved, without making it necessary the
5 administration of exogenous GLP-1. More generally, considering the different effects of GLP-1, i.e. insulin secretion stimulation and satiety induction in the early post-prandial phase, the treatment with metformin can be useful in all pathologic conditions involving deficit of insulin secretion in the early post-prandial phase (for example type 2 diabetes, even in normal weight patients) and/or deficit of satiety
10 (for example obesity, even when not associated with diabetes mellitus) and in any other pathology, at present not foreseeable, where the increase of plasma concentration of GPL-1 is required.

Experimental part

A) effect of metformin on GPL-1 secretion

15 The effect of metformine on the secretion of GPL-1 was studied on 20 obese non-diabetic male patients, aged 30-60 years, 10 of whom received metformin 850 mg per os t.i.d. for 14 days, while the remaining 10 patients received no treatment and were used as a control group. GPL-1 secretion is regulated by glycaemia and insulinaemia; since metformin reduces both glycaemia and insulinaemia, in order
20 to verify the direct effect of the compound on hormone secretion and metabolism it was necessary to develop an experimental model wherein glycaemia and insulinaemia were maintained constant and controlled from the outside. The patients underwent an intravenous infusion of regular insulin ($40 \text{ mU/m}^2 \cdot \text{min}$) and glucose, and glucose infusion rates were adjusted on the basis of glycaemia in

samples of arterialised venous blood drawn every 5 minutes, in order to maintain glycaemia at 100 mg/dl (euglycemic hyperinsulinemic clamp), according to a technique described by DeFronzo et al. 1979. This procedure suppresses endogenous insulin secretion, and allows to maintain glycaemia and insulinaemia constant. After 90' from the beginning of the clamp, glucose (50 g) was administered orally, maintaining glycaemia constant by adjusting the glucose infusion rate accordingly. The circulating concentration of the active forms of GPL-1 (GLP-1[7-36]amide and GLP-1[7-37]) was measured at 0, 30, 60, and 90 minutes from the oral glucose load. This test was performed at the beginning of the study and after two weeks (at the end of metformin therapy for the active treatment group).

Metformin does not modify the basal concentration (i.e. those not stimulated by oral glucose administration) of GPL-1 (mean \pm SD after treatment in the active treatment group : 151 \pm 70 versus 132 \pm 56 pg/ml for GLP-1[7-36]amide, and 17 \pm 12 versus 19 \pm 15 for GLP-1[7-37]; p=NS at Student's paired t test). The treatment with metformin determined a relevant increase of GPL-1 levels after the oral glucose load: the incremental area under the curve (IAUC) increases from 2430 \pm 2781 to 10151 \pm 5058 pg*min/ml for GLP-1[7-36]amide and from 232 \pm 382 to 762 \pm 644 for GLP-1[7-37] (p<0.05 at Student's paired t test) in the active treatment group, while no significant variation is observed in the control group.

It is therefore clear, in the light of the above reported data, that orally administered metformin increases the plasma levels of the active forms of GPL-1 after an oral glucose load, without modifying the basal hormone concentration.

The here demonstrated action of metformin on an endocrine system (GPL-1) involved in the regulation of satiety can suggest a wider use of the compound also on non-diabetic obese patient therefore beyond its present use (type 2 diabetes). Moreover, since GPL-1 is a factor capable of stimulating insulin secretion it can be expected that metformin, through the stimulation of GLP-1, could have a stimulating effect on insulin secretion in the early post-prandial phase. This mechanism of action, ignored up to now, can be an hint to modify therapeutic treatment: metformin, in fact, should no more be considered as a molecule acting on insulin resistance only, and therefore especially suitable for obese diabetes patients, but it is a molecule with a peripheral effect (on insulin sensibility) and an effect, through GPL-1, on insulin secretion (stimulated in the early post-prandial phase).

B) Metformin inhibiting effect on the enzyme DPP-IV

To determine the inhibiting activity of metformine o DPP-IV its effect on the degradation of GLP-1(7-36) amide in vitro was studied by using a pool of plasma from voluntary human donors and in a buffer solution containing DPP-IV.

The plasma was collected from 11 healthy volunteers (6 men, 5 women) slim (body mass index $<27 \text{ kg/m}^2$); with normal glucose tolerance, aged 25-42 years. The blood samples where collected at 8.30 in the morning, after overnight fast, in 10 ml ampoules containing EDTA and 500 UI of kallicrein; the plasma was immediately separated by centrifugation at 4°C. Samples of 1 ml of plasma were incubated for 30' at 37°C with 420 pg of GLP-1[7-36]amide in 0.1 M Tris HCl (pH 8) and with different concentrations (from 0 to 0.5 µg/ml) of metformin. The reaction was stopped after 30' by adding 1 ml trifluoroacetic acid 0.1% and the

samples were extracted on Sep-Pak C18 columns eluted with acetonitrile in trifluoroacetic acid 0.1%. The eluates were lyophilised and stocked at -80°C .

420 pg/ml GPL-1[7-36]amide in Tris HCl 0.1 M 8pH8) were incubated with 0.06 U/ml of DPP-IV from pig kidney (Sigma, St. Louis. USA) in the presence of
5 different concentrations (0 – 0.5 $\mu\text{g/ml}$) of metformin for 0 – 30' at 37°C . The samples were submitted to the same procedures described in the previous experiment.

The concentration of GPL-1(7-36)amide was measured (RIA) in the collected samples.

10 The concentration of GPL-1(7-36) amide measured in the samples at tempo 0, in the absence of metformin, was 356 ± 21 pg/ml (theoretic 440 pg/ml) with a recover of 81%. The addition of metformin up to the highest concentration did not modify such concentration at time 0, showing that the metformin does not interfere with the laboratory determination of GLP-1[7-36]amide concentration. After an
15 incubation of 30' at 37°C , GPL-1[7-36]amide concentration in serum decreases of 42% when compared to time 0. Metformin 0.1 and 0.5 $\mu\text{g/ml}$ markedly inhibits such degradation in a dose-dependent manner; at 0.5 $\mu\text{g/ml}$ metformin inhibits the degradation of GPL-1[7-36]amide almost totally. Similar results were obtained in the buffer solution containing DPP-IV.

20 The above reported data show that the inhibition of GLP-1 degradation caused by metformin is at least partly due to an inhibition of the activity of DPP-IV. That means that the action of metformin on the plasma concentration of GLP-1 is due, at least partially, to the inhibition of the hormone degradation by DPP-IV.

In this particular case the increase of the plasma concentrations of GPL-1 due to the inhibition of the DPP-IV activity can be useful in the treatment of different metabolic disorders as type 2 diabetes and obesity; in fact GLP-1 stimulates the secretion of insulin, reducing glycaemia, and at the same time it inhibits food intake inducing satiety. Moreover, as stated above, considering the different actions of this enzyme, the possibility of inhibiting its activity can be helpful for treating other pathologies where it can be useful to increase the concentrations of NPY, PYY or other possible hormones inactivated by DPP-IV. Moreover, the inhibition of the enzyme can prove useful in the treatment of pathologies of the immune system where the inhibition of CD26 activity is required.

As already said above metformin can be administered in the pharmaceutical forms commonly used and therefore in combination with the common excipients already used for the preparations of such forms, for example in the form of tablets.

The doses normally administered for the therapeutic treatment of the above said pathologies are comprised between 1000 and 2500 mg/die. A typical protocol of administration is for example a tablet containing 850 mg of metformine three times a day before breakfast, lunch and dinner.

CLAIMS

- 1 1. Use of metformine for the preparation of pharmaceutical composition useful to
2 inhibit the enzyme dipeptidyl peptidase IV.
- 1 2. Use according to Claim 1 wherein the pharmaceutical compositions are useful
2 to regulate the concentration of hormones and neuropeptides which are
3 inactivated by the enzyme dipeptidyl peptidase IV.
- 1 3. Use according to Claim 1 wherein the pharmaceutical compositions are useful
2 to regulate the immunity functions modulated by CD26.
- 1 4. Use according to Claims 2 wherein the hormone whose concentration is
2 regulated is GLP-1.
- 1 5. Use according to Claim 2 wherein the neuropeptides whose concentration is
2 regulated are the peptide YY and the neuropeptide Y.
- 1 6. Use according to claims 1 – 5 wherein the pharmaceutical composition consists
2 of metformine and the usual carriers and excipients used for the preparation of
3 oral forms.
- 1 7. Use according to Claim 6 wherein the pharmaceutical composition is in the form
2 of tablets.
- 1 8. Method for inhibiting the activity of enzyme DPP-IV wherein a quantity of
2 metformine comprised between 500 and 850 is administered to the patients 2-3
3 times a day.
- 1 9. Method for increasing the concentration of endogenous GLP-1 wherein a
2 quantity of metformine comprised between 500 and 850 is administered to the
3 patients 2-3 times a day.

1 10. Method according to claim 9 wherein the patient is an obese non diabetic
2 subject.

1 11. Method according to Claim 9 wherein the patient is a diabetic slim or normo-
2 weight subject.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01849

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/155 A61P3/04 A61P3/10 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CHEM ABS Data, EMBASE, EPO-Internal, WPI Data, PAJ, BIOSIS, CANCERLIT, AIDSLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHEEN A.J. ET AL: "'About some non-conventional uses of metformin !. A PROPOS DE QUELQUES UTILISATIONS NON CONVENTIONNELLES DE LA METFORMINE." MEDECINE ET HYGIENE, (1997) 55/2173 (1492-1494). , XP000921039 figure 1 page 1492, column 2, paragraph 2 -page 1493, column 1, paragraph 4 --- -/--	1-11



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 June 2000

Date of mailing of the international search report

20/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Cielen, E

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01849

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 129, no. 17, 26 October 1998 (1998-10-26) Columbus, Ohio, US; abstract no. 211523, LUGARI, R. ET AL: "Effects of metformin on intestinal and pancreatic endocrine secretion in type 2 (non-insulin-dependent) diabetes" XP000920928 abstract the whole document & FRONT. DIABETES (1998), 14(MOLECULAR AND CELL BIOLOGY OF TYPE 2 DIABETES AND ITS COMPLICATIONS), 161-163 ,	1-9,11
X	--- SCHEEN A.J.: "'How to treat... A non - obese patient with diabetes mellitus type 2!. COMMENT JE TRAITE... UN PATIENT DIABETIQUE DE TYPE 2 NON OBESE." REVUE MEDICALE DE LIEGE, (1994) 49/3 (121-122). , XP000921038 page 122, paragraph 2 - paragraph 3 ---	1-9,11
X	JACKSON R A ET AL: "Mechanism of metformin action in non-insulin-dependent diabetes" DIABETES, (1987 MAY) 36 (5) 632-40. , XP000921037 abstract table 1 --- -/--	1-9,11

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01849

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PAOLISSO G (REPRINT) ET AL: "Effect of metformin on food intake in obese subjects"</p> <p>EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, (JUN 1998) VOL. 28, NO. 6, PP 441-446. PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND. ISSN: 0014-2972., vol. 28, no. 6, June 1998 (1998-06), pages 441-446, XP000218679</p> <p>UNIV NAPLES 2, DEPT GERIATR MED & METAB DIS, SERV ASTANTERIA MED, PIAZZA MIRAGLIA 2, I-80138 NAPLES, ITALY (Reprint); UNIV NAPLES 2, INST ENDOCRINOL, I-80138 NAPLES, ITALY</p> <p>abstract</p> <p>page 441, column 1, paragraph 1 -column 2, paragraph 2</p> <p>page 442, column 1, paragraph 3</p> <p>page 445, column 1, paragraph 2 - paragraph 3</p> <p>page 445, column 1, paragraph 5 -column 2, paragraph 2</p> <p>page 446, column 1, paragraph 2 - paragraph 3</p> <p>---</p>	1,2,5-10
X	<p>ROURU J ET AL: "Anorectic effect of metformin in obese Zucker rats: lack of evidence for the involvement of neuropeptide Y."</p> <p>EUROPEAN JOURNAL OF PHARMACOLOGY, (1995 JAN 24) 273 (1-2) 99-106. , XP000920931</p> <p>abstract</p> <p>figure 4</p> <p>page 104, column 2, paragraph 3 - paragraph 4</p> <p>page 105, column 1, paragraph 2 - paragraph 3</p> <p>page 106, column 1, paragraph 2</p> <p>---</p>	1,2,5,6
X	<p>REYNOLDS J.: "Martindale - The Extra Pharmacopeia. Edition 31"</p> <p>1997 , ROYAL PHARMACEUTICAL SOCIETY , LONDON, GB XP002141210 224540</p> <p>page 357, column 2, paragraph 2 -column 3</p> <p>---</p> <p>-/--</p>	8,9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01849

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 57634 A (SMITH STEPHEN ALISTAIR ;SMITHKLINE BEECHAM PLC (GB)) 23 December 1998 (1998-12-23) abstract page 1, line 1 - line 8 page 1, line 34 -page 2, line 25 page 4, line 10 - line 17 page 6, line 12 - line 32 claims 1,2,14,15,17,20,21 ----	1-9
P,X	STEFANOVIC V ET AL: "Reversal of increased lymphocyte PC-1 activity in patients with type 2 diabetes treated with metformin." DIABETES METAB RES REV, (1999 NOV-DEC) 15 (6) 400-4. , XP000921051 abstract page 401, column 1, paragraph 4 -column 2, paragraph 1 page 402, column 2, paragraph 4 table 4 page 404, column 1, paragraph 3 ----	1,8
P,X	DE 299 09 210 U (PROBIODRUG GES FUER ARZNEIMITT) 9 September 1999 (1999-09-09) page 1, paragraph 1 - paragraph 3 page 2, paragraph 1 - paragraph 2 page 4, paragraph 2 - paragraph 4 page 9, paragraph 3 -page 10, paragraph 2 page 11; table 1 ----	1,2,4,8, 9,11
A	TANAKA SUMIKO; MURAKAMI TAKANORI; HORIKAWA HIROSHI; SUGIURA MASAKI; KAWASHIMA KEISUKE; SUGITA TAKAHISA : "Suppression of arthritis by the inhibitors of dipeptidyl peptidase IV." INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY, vol. 19, no. 1, 1997, pages 15-24, XP000921050 abstract page 16, column 1, paragraph 1 - paragraph 2 page 20, column 1, paragraph 1 page 22, column 2, paragraph 2 - paragraph 3 ----	1,3
A	WO 98 19998 A (CIBA GEIGY AG ;VILLHAUER EDWIN BERNARD (US)) 14 May 1998 (1998-05-14) abstract page 1, paragraph 2 page 18, paragraph 3 page 19, paragraph 2 -page 20, paragraph 1 ----	1-11
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01849

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HOLST JENS J; DEACON CAROLYN F :</p> <p>"Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. "</p> <p>DIABETES ,</p> <p>vol. 47, no. 11, November 1998 (1998-11),</p> <p>pages 1663-1670, XP000853619</p> <p>abstract</p> <p>page 1663, column 2, paragraph 1</p> <p>page 1664, column 2, paragraph 2 -page</p> <p>1665, column 2, paragraph 2</p> <p>page 1666, column 1, paragraph 5 -column</p> <p>2, paragraph 2</p> <p>page 1667, column 1, paragraph 2 -column</p> <p>2, paragraph 1</p> <p>page 1667, column 2, paragraph 3</p> <p>page 1668, column 1, paragraph 2</p> <p>-----</p>	1-11

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 00 01849

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-8 relate to a use and method, defined by reference to the parameter "to inhibit the enzyme dipeptidyl peptidase IV". Moreover, claims 2,4-7 relate to the parameter "to regulate the concentration of hormones and neuropeptides which are inactivated by the enzyme dipeptidyl peptidase IV", claim 3 relates to a use defined by reference to the parameter "to regulate the immunity functions modulated by CD26", claims 4,6,7,9-11 relate to the parameter "regulation of the concentration of GLP-1" and claims 5-7 relate to the parameter "regulation of the concentration of peptide YY and neuropeptide Y". Since the pharmacological action of this compound is not well-defined, the use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the diseases specified in claims 10 and 11, namely obesity in non-diabetic subjects and diabetes in slim or normoweight subjects, with due regard to the general idea underlying the application.

Claims searched partially: 1-11.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/01849

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9857634 A	23-12-1998	AU 8539398 A EP 0996444 A NO 996266 A	04-01-1999 03-05-2000 17-12-1999
DE 29909210 U	09-09-1999	DE 19823831 A AU 4370999 A DE 29909208 U DE 29909211 U WO 9961431 A	02-12-1999 13-12-1999 09-09-1999 23-09-1999 02-12-1999
WO 9819998 A	14-05-1998	AU 5318498 A BR 9714130 A CN 1236361 A CZ 9901615 A EP 0937040 A NO 992028 A PL 332777 A	29-05-1998 29-02-2000 24-11-1999 11-08-1999 25-08-1999 28-04-1999 11-10-1999

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/155, A61P 3/04, 3/10, 37/00	A1	(11) International Publication Number: WO 00/53171 (43) International Publication Date: 14 September 2000 (14.09.00)
(21) International Application Number: PCT/EP00/01849 (22) International Filing Date: 3 March 2000 (03.03.00) (30) Priority Data: FI99A000040 5 March 1999 (05.03.99) IT FI99A000215 19 October 1999 (19.10.99) IT (71) Applicant (for all designated States except US): MOLteni L. E C. DEI FRATELLI ALITTI SOCIETA' DI ESERCIZIO S.P.A. [IT/IT]; Strada Statale 67 - Tosco-Romagnola, Località Granatieri, I-50018 Scandicci (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): MANNUCCI, Edoardo [IT/IT]; Via Dante 5, I-59100 Prato (IT). ROTELLA, Carlo, Maria [IT/IT]; Via S. Martino 6, Loc. Grassina, I-50012 Bagno A Ripoli (IT). OGNIENE, Agostino [IT/IT]; Piazza Villamagna 5, I-50012 Bagno A Ripoli (IT). (74) Agent: GERVASI, Gemma; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: USE OF METFORMIN IN THE PREPARATION OF PHARMACEUTICAL COMPOSITIONS CAPABLE OF INHIBITING THE ENZYME DIPEPTIDYL PEPTIDASE IV (57) Abstract <p>The use of metformin in the preparation of pharmaceutical compositions useful for inhibiting the enzyme dipeptidyl peptidase IV, in particular for increasing the plasma concentration of Glucagon-Like Peptide-1, is described.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		